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Wine bottle colour and oxidative spoilage: whole bottle light exposure experiments under controlled and uncontrolled temperature conditions

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Abstract

Exposure of a Chardonnay wine to light from a mercury vapour lamp under controlled temperature conditions showed that colour enhancement was dependent on bottle colour. The increase in colouration was Antique Green < French Green < Arctic Blue < Flint, in agreement with the transmission characteristics of each bottle type. Xanthylium pigments were identified as one component contributing to the observed enhancement of colour. The presence of oxygen was shown to be a critical factor to initiate the formation of these xanthylium pigments during light exposure. Without temperature control, wine colour development was highest in Antique Green and lowest in Flint. This alternate order reflects the ability of the darker bottles to retain heat longer than lighter coloured ones as confirmed by surface temperature decay rates. Specific pigments contributing to the wine colour enhancement in uncontrolled temperature/light exposure experiments could not be identified, although tentative evidence was obtained for the presence of flavan-3-ol based compounds. The different bottle glass surfaces did not influence the rate of loss of dissolved oxygen or oxidation of ascorbic acid. The potential to develop the results obtained in this study to identify markers for light and/or temperature exposure of white wines is discussed.

Keywords

White wine; colour enhancement; light exposure; bottle colour; oxygen; bottle surface effects

1. Introduction

A range of factors can impinge on the selection of bottle colour and weight for the storage of wine. Consumer acceptance is one factor where market forces may drive the use of lighter coloured glass bottles over the traditional dark green or brown glass. Recycling costs may form part of the decision making process and is likely to become a significant factor as carbon accounting becomes a more prominent issue in trade and marketing. Carbon costs can influence the selection of both the colour and weight of the bottle (WRAP, 2012). Tradition may also be a determining factor. The special shape and colour of the *Bocksbeutel* used in Franken (Germany) is a good example.

Storage conditions, especially for white wine, may also influence the choice of bottle colour and bottle weight. Exposure to light and elevated temperature can generate the production of increased colour and off-odours. The influence of simulated solar radiation on the redox potential of Champagne wines held in glass cells constructed from Champagne bottle glass and an 'anti-UV' glass showed that radiation less than 450 nm was required for the generation of a light-struck effect, known as *goût de lumière* (Maujean, Haye & Feuillat, 1978). Extending this study, (Maujean & Seguin, 1983)) identified the off-odours as degradation products of sulfur-containing amino acids, a result reinforced in a study of still wines from Italy, Spain and Slovenia (Mattivi, Monetti, Vrhovšek, Tonon & Andrès-Lacueva, 2000).

Simulating conditions that may occur in retail outlets, (Dozon & Noble, 1989) examined the impact of fluorescent lighting on aroma profiles of both sparking and still white wines stored in green and clear glass bottles. Green glass provided more protection against negative effects

of light, requiring considerably longer exposure times before detectable sensory differences were observed.

While off-odour production due to light exposure has been examined in some detail, there has only been minimal work on the link between light impact and colour enhancement in white wine. When *fino* wines stored in transparent and topaz bottles were exposed to radiation from a xenon lamp at 25ºC, a higher absorbance at 420 nm was observed after 45 days for wines in the darker topaz bottles (Benítez, Castro & Barroso, 2003). Exposure of a Sauvignon Blanc wine in different coloured bottles (Antique Green, Classic Green, French Green and Flint) to sunlight for 70 days also showed that more colour development occurred in the dark green (Antique Green, Classic Green) bottle than in Flint or French Green (Maury, Clark & Scollary, 2010). In this study by Maury et al. (2010), the bottles were stored externally and subjected to a wide variation in both maximum and minimum values of temperature. On the other hand, lower colour development was observed when an alcohol beverage made from orange juice was stored in brown glass with clear glass giving the highest colour and green glass intermediate (Selli, Canbaş & Ünal, 2002).

In a more recent study, radiation of less than 400 nm was focussed on a Chardonnay sample held in a cuvette with sections of glass bottles used as a filter simulating bottle exposure (Dias, Smith, Ghiggino & Scollary, 2012). When the experiments were performed at a constant temperature of 45ºC, colour enhancement depended on bottle colour with the order being Flint > Arctic Blue > French Green > Antique Green. This order is the reverse of that observed by Benítez et al (2003) and Maury et al. (2010).

This work was undertaken to resolve the influence of light and temperature on colour enhancement in white wine stored in bottles widely used in the wine industry. Exposure experiments were performed in a light box for which the temperature could be controlled. In addition to the environmental factors of light and temperature, the impact of oxygen and the potential for the glass surface to contribute to oxidative processes were also examined.

2. Material and Methods

2.1 Reagents

All items of glassware and plasticware were soaked for at least 16 h in 10% (v/v) nitric acid (BDH, AnalaR) and then rinsed with copious amounts of grade 1 water (ISO3696). Solutions and dilutions were prepared using Grade 1 water. Ethanol (AR grade, >99.5%) was purchased from Ajax Fine Chemicals (Australia). (+)-Catechin (Sigma, 98%) and ascorbic acid (Sigma, 99%) were used without further purification.

2.2 Wine samples

Chardonnay samples [ethanol: 13.0% (v/v)] were purchased commercially in 10 L bag-in-box containers. The wines were enriched with (+)-catechin at 100 mg/L. This flavan-3-ol concentration was chosen to represent a white wine produced from heavily extracted grapes and would be at the higher end of flavan-3-ol concentration in white wine. This is in line with systems used in other light exposure studies (Clark, Dias, Smith, Ghiggino & Scollary, 2011; Dias, Smith, Ghiggino & Scollary, 2010; Maury et al., 2010).

2.3 Glass bottles

Wine bottles (Claret punted, 750 mL) were used in this research. The bottles are designated here by their trade names (Flint, Arctic Blue, French Green and Antique Green) to describe

their colour. Both traditional weight and newer light weight bottles were used. A detailed description of the transmission characteristics for each bottle type can be found elsewhere (Clark et al., 2011; Dias et al., 2010).

2.4 Large scale irradiation setup

Wine bottles of different colours were filled to a volume of 740 mL with the Chardonnay wine enriched with 100 mg/L (+)-catechin. The headspace of each bottle was flushed for 2 min with nitrogen. The bottles were sealed with screw cap closures and placed into the wine bottle holder (see Supplementary Figure S1). Each bottle was placed at a slight angle in a circular manner in the holder to ensure that each bottle received an equivalent amount of light from the light source. The light box was designed to hold up to 8 bottles in any one experiment.

The bottle holder was then placed in a 'light-tight' box (see Supplementary Figure S2). The light source used in the irradiation experiments was a MegaRay® mercury vapour, self ballasted, 160 W (high UVA and UVB flood lamp) placed approximately 40 cm above the bottles. The UV output at the working distance was 150 $\mu W/cm^2$, equivalent to full sun at midday. A thermocouple allowed measurement of the ambient temperature inside the chamber and bottle surface temperature during the course of the irradiation.

An exhaust fan was placed inside the chamber allowing for a constant temperature of 38 ± 3°C for all bottles. The ambient temperature was 30 ± 2°C. The entire system was controlled by a 24 h timer. Exposure times of 16 h were used followed by 8 h without light, the latter to simulate night time. Normally, this pattern of exposure/non-exposure was applied for 18 days with daily aeration.

2.5 Spectral and LC analyses

The absorbance measurements were recorded at 440 nm and spectra recorded from 200 to 600 nm using a Cary Bio50 UV/Vis spectrophotometer. CIELab measurements were conducted on a Shimadzu (Kyoto, Japan) UV-1700 UV/visible spectrophotometer with UVPC Color Analysis software (version 3.00). CIELab parameters were as described within (Clark, Vestner, Barril, Maury, Prenzler & Scollary, 2010).

Liquid chromatography was conducted with an ultra high performance liquid chromatography (UHPLC) system consisting of a Waters (Milford, MA) Acquity binary solvent manager connected to a sample manager and a PDA detector all run by Empower^2 chromatography manager software. The column was a Waters Acquity BEH C18 (2.1 x 50 mm) with 1.7 μm particle diameter. The operating conditions were as specified within (Clark et al., 2010).

LC-MS studies conducted on an Agilent 1200 series Triple Quadrapole (6410) HPLC-MS. The column and LC elution gradient was as described above for the UHPLC experiments, except for an injection volume of 5 μL and 0.2 %(v/v) formic acid replaced acetic acid as the solvent buffer. The MS was operated at 350°C, gas flow of 9 L/min, nebulizer at 40 psi, and capillary at 4 kV. LC-MS analyses for the samples were carried out in both the negative and positive ion modes with the fragmentor at 80V and scanning performed between 100-800 *m/z*.

2.6 Measurement of dissolved oxygen and headspace oxygen

Measurement of dissolved and headspace oxygen was performed using a Presens oxygen analyser (Fibox 3 LCD v7) with pre-calibrated PSt3 sensors. A Chardonnay sample was

prepared by adding 100 mg/L of (+)-catechin and allowing the wine to equilibrate in darkness for 2 hours. The wine (approximately 740 mL with a headspace volume of ~27 mL) was added to 6 Flint bottles (traditional weight) with each bottle having one oxygen sensor just above the punt (dissolved oxygen measurements) and another in the bottle neck (headspace measurements). The wine in three bottles was flushed with carbon dioxide for 5 min and sealed with a screw cap ('*low*' oxygen) while the other three bottles were simply capped ('*high*' oxygen). All bottles were stored for 7 days to equilibrate before irradiation experiments. Irradiation was conducted as described in Section 2.4, but without daily aeration for the '*low*' oxygen sample. The '*high*' oxygen bottles were aerated each day for 1 h, re-capped and allowed to equilibrate for 30 min prior to irradiation.

2.7 Bottle surface effects

For the dissolved oxygen decay experiments, an oxygen sensor was placed in the lower section of 3 Flint and 3 Arctic Blue (traditional weight) bottles and left overnight in air to equilibrate. The next morning, the Chardonnay wine was added to each bottle to just above the level of the sensor (wetting step). This was removed after 30 min and the bottles were filled to the top (no headspace) with a fresh sample of the same wine. Dissolved oxygen measurements were made at time zero (that is, immediately after filling) and at 10 min intervals for the first 110 min and then at 30 min intervals up to 320 min with the final measurement being made the next morning (16 h after the experiment began). One Flint and one Arctic Blue bottle were filled with the Chardonnay and a temperature probe placed in the bottles to allow accurate recording of the temperature during the experiment.

For the ascorbic acid decay experiments, ascorbic acid (100 mg/L) was added to a model wine system (Barril, Clark & Scollary, 2008). This solution was then added to Flint, Arctic

Blue, French Green and Antique Green heavy/light weight bottles in triplicate (after flushing with nitrogen) and the bottles stored away from light at room temperature. The concentration of ascorbic acid was monitored on four occasions over a six day period by titration with 2,6-dichlorophenolindophenol (Ough & Amerine, 1988).

2.8 Statistics

The uncertainty associated with the light irradiation experiments was assessed by triplicate irradiation of wine samples in Flint glass bottles (heavy weight) under high oxygen regimes. The replicate difference absorbance spectra after irradiation for 17 days are shown in Supplementary Figure S8. The relative standard deviation between the replicates for the absorbance at 480 nm was 4.1 % and the average relative standard deviation over the wavelength range 700-380 nm was within 10%.

3. Results and discussion

3.1 Exposure of wine in different glass bottles to radiation under controlled temperature conditions

Arctic Blue, French Green and Antique Green, both heavy and light weighted bottles, and heavy weighted Flint were filled with Chardonnay to which (+)-catechin (100 mg/L) had been added, headspace flushed with N_2 and sealed. All bottles were irradiated for 18 days (16 h light, 8 h darkness) at a temperature inside the lightbox of 30 ± 2°C. Bottle surface temperatures, however, were recorded to reach 38 ± 3°C through absorption of heat during the exposure to light. Bottles were aerated during the sampling required for absorbance measurements. Difference absorption spectra were recorded periodically from Day 1 to Day 18.

Two controls were also established for each bottle type and were stored in the dark for 18 days at room temperature. One control was prepared with the Chardonnay wine without added (+)-catechin and the second control consisted of the Chardonnay with added (+)-catechin. Negligible pigmentation development was observed for these two control samples, noting however, that the samples were stored at an ambient temperature of 25^{o}C, rather than the light exposure study temperature of 30^{o}C (bottles reaching 38 ± 3^{o}C).

The difference absorption spectra for the samples in the different bottle types exposed to light over the 18-day period are presented in Figure 1. It is apparent from these difference spectra that there are some commonalities between bottle types and also some noteworthy differences. Exposure of the wine resulted in the growth of two absorption peaks. One is centred around 380 nm that tails into the visible region and the other is around 450 nm (compared to 480 nm in small scale irradiation experiments) (Dias et al., 2012). The relative absorbance of these two peaks is dependent on bottle colour. Visually, the colour intensity of the wine after exposure decreased in the order: Flint > Arctic Blue > French Green > Antique Green.

The wine stored in the Antique Green bottle showed a marked increase in the 380 nm absorbance, reaching 0.13 absorbance units after 18 days exposure. The absorbance at 450 nm only achieved a value of 0.063 absorbance units by the end of the experiment. The relative height of the two peaks was consistent throughout the time course of the experiment (Fig 1a). There is essentially no difference between the absorbance profiles for the heavy (Fig 1a) and light (Fig 1b) weighted Antique Green bottles.

Two peaks were also observed in the difference spectra for the wine stored in French Green bottles (Fig 1c) and again, there is essentially no difference between the bottles of different weight (Fig 1c and 1d). There are, however, some major differences when compared with that observed for the wine in Antique Green bottles: the absorbance values are higher for French Green (A_{380} = 0.20 and A_{450} = 0.13) reflecting the higher observed colour of the exposed wine. The ratio of the absorbance values for the two peaks (A_{380}/A_{450}) is less with the French Green bottle than with Antique Green (compare Figure 1a and Figure 1c) and there is a slight lag period in the growth of the peak at 380 nm, but after 3 - 4 days exposure, the increase in absorbance is faster than for the 450 nm peak. The impact of bottle colour on the difference absorption spectra for the light exposed samples is obvious when the results for the Arctic Blue bottles are examined. The spectra (Figure 1e) showed that the ratio of absorbance values for the two peaks (A_{380}/A_{450}) are much closer to unity than observed with Antique Green and French Green and the A_{450} values are higher than with Antique Green and French Green, reflecting the observed darker colour of the exposed wine after 18 days. Noticeably higher absorbance values were found with the light weighted bottle compared with the heavy weighted bottle at 380 nm (heavy: 0.16; light: 0.21) and 450 nm (heavy: 0.14; light: 0.19). Figure 1g presents the spectral changes for the heavy weighted Flint bottle over the 18 day exposure period. The two peaks at 380 nm and 450 nm are similar in intensity to that observed for both Arctic Blue (Heavy and Light weighted) bottles and considerably higher in value in comparison with all other bottle types used in this experiment.

These data represent a unique set of results that has significant implications for the careful storage of wine. Clearly, and in accordance with our previous iron (III) tartrate photoactivation study (Clark et al., 2011) and small scale irradiation studies (Dias et al., 2012), there was less colour development in the darker bottles than in the Arctic Blue and

Flint. Of importance, however, was the ability of the Arctic Blue bottle to provide less colour development than the Flint bottle, despite the similar transmission spectra of these bottles (Dias et al., 2010). Further, as we have previously observed, colour changes were observed in all bottle types, implying that bottle colour does not provide total protection from light-induced changes (Dias et al., 2012). The bottle weight did have a minor impact on colour production although this was not as critical as bottle colour. The influence of bottle colour on the absorbance profiles in Figures 1 a-g suggests that a series of complex and possibly competing reactions are occurring. This investigation forms the basis of a longer-term study on the chemistry of the reaction sequences that are occurring.

3.2 Aroma and colour profiles of wines irradiated with light at constant temperature

Both aroma and colour changes were observed during the course of light exposure. A preliminary aroma sensory assessment was performed on the Chardonnay samples after 18 days exposure to light. The most common descriptor identified was acetaldehyde together with caramel, almond, quince and acetic acid, indicating that oxidative processes were significant as a consequence of light exposure in oxidative conditions.

To obtain a better assessment (quantitative and descriptive) of the colour variation for the wine stored in different bottles for 18 days were analysed using CIELab measurements. The data in Table 1 are in accordance with the general visual assessment of the wines. It is apparent that the greatest colour intensity was observed for Flint (heavy): $L^* = 85.83$ and the least colour intensity was observed for Antique Green (heavy): $L^* = 97.05$. There is a significant shift to red colouration in the order: Antique Green < French Green < Arctic Blue < Flint, with the same trend also apparent for shift to yellow colouration. There is more red and yellow colouration (Table 1) in the light weight Arctic Blue bottle than in the

corresponding heavy weight bottle. This difference between the two bottle weights is more apparent in this whole bottle study than in the small scale irradiation study described elsewhere (Dias et al., 2012). It must be noted that the exposure time is much longer in this whole bottle study than in the small scale study. When the small scale study using Arctic Blue was repeated for 16 h compared with 6 h in (Dias et al., 2012), a marked difference in the final absorbance was noted (see Supplementary Figure S3). It should also be noted that only minimal spectral changes were observed in the first 4 days of the Arctic Blue whole bottle exposure (Figure 1e), suggesting that there may well be an induction period prior to the onset of more rapid colour enhancement.

3.3 Identification of pigments formed as a consequence of exposure to light under controlled temperature conditions.

Figure 2 presents the UHPLC chromatogram with detection at 440 nm for the Chardonnay sample containing 100 mg/L added (+)-catechin after 18 days exposure to light in heavy and light weighted Arctic Blue bottles. Chromatograms for samples in other bottles used in this study can be found in Supplementary Figure S4. By comparison with a standard xanthylium cation prepared from (+)-catechin and glyoxylic acid (Clark, Prenzler & Scollary, 2007), the peaks at 3.11, 3.26 and 3.43 min (labelled with '*') could be assigned to different isomeric forms of the xanthylium cation while the peak at 4.34 min corresponds to the ethyl ester derivative (Clark et al., 2007). The highest production occurs in the Flint (heavy) bottle and the lowest amount is formed in the Antique Green samples. This is in accord with the colour analysis described in Table 1. Xanthylium pigment production is more extensive in the lightweight Arctic Blue bottle than in the heavy weight Arctic Blue bottle (Figure 2), again in accord with the colour data (Table 1).

Further confirmation of the presence of xanthylium pigments was obtained by LC-MS. There are several linkage isomers that can be formed and a characteristic pattern of peaks is commonly observed. All exposure study samples were analysed by LC-MS with data consistent with the assignment of pigment peaks as xanthylium cations in their acid and ethyl ester forms. The acid forms had parent ion signals at 617 *m/z* and the ethyl ester form was 645 *m/z*. Fragmentation ions at 465 and 152 *m/z* for the acid forms and 493 *m/z* for the ester forms were consistent with ion fragments reported previously for these compounds (Labrouche, Clark, Prenzler & Scollary, 2005). It is important to mention that xanthylium cations are not the only pigments generated but that the general increase in the chromatographic baseline, and other unknown peaks, mirrored the trends of the xanthylium cation concentration. Also, the production of xanthylium cation pigments in the presence of wine non-flavonoids, such as caffeic acid, is known to generate a chromatographically unresolved range of polymeric pigments that contribute brown colour to model wine systems (George, Clark, Prenzler & Scollary, 2006).

3.4 Bottle exposure experiments under uncontrolled temperature conditions

This experiment was essentially identical to that described above except that temperature was not controlled and irradiation was carried out over three days only without aeration. Samples of Chardonnay with added (+)-catechin (100 mg/L) were added to the Flint, Arctic Blue, French Green and Antique Green light weighted bottles. These bottles were irradiated for 3 days (16 h light, 8 h darkness). During the periods of light exposure, the temperature of the glass surface reached at least 80^{o}C. The short time period (3 days compared with 18 days under controlled temperature conditions) was sufficient for visual perception of pigmentation.

Figure 3 presents the difference absorption spectra for samples in each bottle type after 3 days exposure (see Supplementary Figure S5). Growth of a peak around 380 nm tailing into the visible region is obvious, with the largest increase occurring for the Antique Green light weight bottle. This is exactly the reverse of the results obtained when the temperature was controlled. There is no growth of a specific peak at 450 nm, as was observed when the same wine was exposed to light under controlled temperature conditions. Rather, colour development is a consequence of the spectrum tailing into the visible region.

UHPLC chromatograms at 440 nm for the Chardonnay samples in the four light weighted bottles after 3 days exposure to light without temperature controlled are presented in Figure 4. These chromatograms are significantly different to those obtained for samples exposed to light under controlled temperature conditions (see Figure 2). There is a single peak at 3.6 min that is dominant and common to all samples, as well as a general increase in the baseline of the chromatograms. LC-MS measurements indicated a *m/z* value of 563 (positive ion mode) for this peak. Its full structural identity has not yet been determined. There is no evidence for the presence of xanthylium pigments in the uncontrolled temperature samples. The increase in the background of the chromatogram in Figure 4 suggests that polymeric pigments may have developed during this exposure experiment without temperature control. The chromatographic conditions used did not allow resolution of the components that contribute to the increase in the baseline.

Preliminary aroma profiling on the samples after 3 days exposure to light without temperature control indicated that in addition to acetaldehyde, honey and kerosene aromas were also present. These latter descriptors may well reflect the formation of odorants such as phenylacetaldehyde (honey aroma), sotolon and 1,1,6-trimethyl-1,2-dihydronaphthalene

(TDN, kerosene aroma), all of which require oxidative processes at elevated temperatures to be formed (Ferreira, Hogg & Guedes de Pinho, 2003).

CIELab measurements were also carried out on these samples (Table 1). In terms of colour intensity, the Antique Green sample is the most intense (L^*: 93.07), in line with the visual assessment of colour. The values for the a^* parameter show that the red colouration is greatest for the Antique Green sample. The b^* parameter indicates that all samples exhibit significant yellow colouration with again the Antique Green sample being the most yellow.

The CIELab measurements (Table 1) for these uncontrolled temperature samples are different to those for the temperature controlled samples. Not only are the colour intensities the reverse order for Antique Green and Flint (the two extremes in these exposure studies), but the green to red and blue to yellow parameters also differ. The different colour parameters are further evidence that different pigmentation processes are occurring between the controlled and uncontrolled temperature samples. Further work is required to establish the basis for this difference with the potential to develop a marker for sample degradation as a consequence of light or temperature exposure.

3.5 *Differentiating between light and temperature effects that result in pigment production*

The outcomes of the experiments performed in this investigation have allowed a separation of temperature effects under equivalent light conditions. Under controlled temperature (30^{o}C for ambient air in a lightbox, 38 ± 3^{o}C for bottle surface temperature) and light conditions, the lighter coloured bottles (Flint and Arctic Blue) show the highest amount of colour development, while minimal colour development occurs in Antique Green bottles. This can be related to the higher levels of low wavelength light that can be transmitted by the Flint and

Arctic Blue bottles. Exposure under the same light regime, but without temperature control, reversed the order of the bottle influence on colour. That is, when ambient air temperature in the light box rose to 80ºC using the light as the heat source, the Chardonnay sample stored in Antique Green bottles showed the most colour development.

Maury et al. (2010) proposed that dark coloured glass absorbs more heat and retains the heat longer, which ultimately drives pigmentation development in darker bottles. In the bottle exposure experiment under controlled temperature conditions, thermocouples were placed in the 'light box' (Supplementary Figure S2), one assessing ambient air temperature, the other attached to the surface of the bottle. Supplementary Figure S6 illustrates the temperature changes recorded on the surface of the bottle over a 24 h measurement period. When the light was turned on, the surface temperature of the bottles increased at much the same rate, but the plateau temperature for Antique Green was some 5ºC higher than for Flint, confirming the proposal of Maury et al. (2010).

The controlled/uncontrolled temperature experiments in this investigation also showed differences in the type of pigments that are formed as a consequence of exposure to light. Xanthylium pigments were identified as a major contributor to colour development in the controlled temperature experiments. On the other hand, the same pigments were not found in the uncontrolled temperature experiments. Xanthylium pigments were found in the uncontrolled temperature light exposure experiments of Maury et al. (2010), but under conditions different to those used in the experiments described here. The exposure periods are quite different, 70 days versus 3 days, and aeration conditions also differ. George et al. (2006) described several factors that can affect the stability of xanthylium pigments, one of which is light exposure. It is plausible that xanthylium pigments may be precursors of other

pigments, forming, breaking down and re-forming over time especially at higher temperatures. Indeed, it has been demonstrated that xanthylium cations can undergo further polymerisation with the production of red pigments (Es-Safi, Cheynier & Moutounet, 2003). This needs to be the subject of further research. It is evident that the exposure experiments performed in this study have opened up the possibility of identifying markers, both pigments and aroma compounds, which could be used to identify the occurrence of wine degradation in storage or transport.

3.6 Influence of molecular oxygen on the light-induced pigmentation development

An assessment of the contribution of molecular oxygen to the light-induced oxidative processes was carried out using Flint glass bottles as this bottle colour showed the largest exposure impact under controlled temperature conditions. The free sulfur dioxide, total sulfur dioxide and ascorbic acid concentrations were 30 ± 1 mg/L (n = 3), 150 ± 10 mg/L (n = 3) and 260 ± 10 mg/L (n = 3), respectively, and were determined as described (Barril, Clark & Scollary, 2012).

Figures 5a and b present the average data for the 3 bottles in '*low*' and '*high*' oxygen category, respectively. The loss of headspace oxygen (HO) and dissolved oxygen (DO) in the '*low*' oxygen case (Figure 5a) is consistent with the consumption of oxygen by various wine components and then depletion as the oxygen was not being replenished. In the '*low*' head space sample the dissolved oxygen (DO) oxygen rapidly decreased to negligible levels by day 8 due to consumption by the wine. The HO value increased slightly and then decreased to negligible levels by day 13. The increase in the headspace oxygen was probably due to transition of some DO to HO possibly as a consequence of the particularly low levels of the headspace value. Progressively this HO redissolved into the wine and was consumed. In fact,

these results are interesting as to our knowledge there are no reports on the movement of the dissolved oxygen to the head space in bottled wine. These results demonstrate this can occur where DO is high and HO oxygen is low and is particularly relevant to bottling operations that are efficient in removing HO oxygen.

For the '*high*' oxygen sample, the HO oxygen remains fairly constant and high (i.e. as expected, as the samples were constantly aerated), whilst the dissolved oxygen reaches very low levels after day 8. Although there were no further changes in the dissolved oxygen concentration, visual inspection of these '*high*' oxygen wines indicated that they became progressively darker from day 10 to day 17. These observations for the '*high*' oxygen wines are in accord with the comments of (Bradshaw, Scollary & Prenzler, 2004) that oxygen may only be required to initiate the reactions that lead to pigmentation. That is, there may be a critical point after which oxygen exposure is not needed for on-going pigmentation development. These data further suggests that, after day 8, the rate of dissolved oxygen consumption by the wine is similar to the rate of dissolution of oxygen into the wine from the headspace, and for this reason no accumulation of dissolved oxygen is evident.

Images of the bottles taken after the irradiation period are shown in Supplementary Figure S7. It is apparent that oxygen is important for the development of pigmentation. Only the oxygenated samples showed a marked increase in pigmentation after exposure to radiation over the 17-day period.

Figure 6a presents the 280 nm UHPLC chromatogram for the '*high*' and '*low*' oxygen samples. The '*high*' oxygen sample shows that more catechin has reacted after 17 days exposure than the '*low*' oxygen sample. The 440 nm UHPLC chromatogram shows the

presence of xanthylium pigments in the '*high'* oxygen sample only (compare Figures 6b and 6c). It is evident from these results that molecular oxygen plays an essential role in the production of pigments during irradiation with light.

3.7 Assessment of the influence of the glass surface on oxidative processes

The surface of glass is well-known to activate some chemical processes and, given the different compositions of glass used to make the bottles that were employed in this project, two experiments were performed to check for surface activity: measurement of the loss of dissolved oxygen on bottling and the decay of ascorbic acid in a model wine system. The experiments were performed to confirm that the results of the light exposure studies were not influenced by bottle surface effects.

3.7.1 Dissolved oxygen decay

This experiment was carried out to compare the rate of loss of dissolved oxygen in wine stored in light weighted Flint and Arctic Blue bottles. Supplementary Figure S8 shows that the rate of loss of dissolved oxygen in the first measurement period is essentially the same for both bottle types and the final DO concentration is the same in both cases. In essence, the data from this experiment indicate that there is no influence of bottle colour, and hence surface chemical composition, on the decay of dissolved oxygen post-bottling.

3.7.2 Decay of ascorbic acid in a model wine system

The results of this experiment (see Supplementary Figure S9) showed that the oxidative loss of ascorbic acid are very similar in all bottle types, again confirming (as with the dissolved oxygen decay) that there is no influence of the surface of the glass bottle on the solution chemistry oxidative processes.

4. Conclusions

The results of this study suggest a pathway for the phenomenon of 'random oxidation' as they demonstrate that both light and oxygen are critical for pigment production. Thus, in situations where the oxygen concentration within a bottle varies, from (for example inefficient inert gassing at specific bottle filling heads in during wine bottling or due to poor closure integrity), an environment is established that will allow pigmentation to develop if the wine is exposed to light. The link between changes in either the dissolved or headspace concentrations and light-induced pigment development provides a much stronger basis for developing criteria to minimise the random oxidation phenomenon. The results also show that in the absence of light, the compositional differences in the different coloured glasses does not have a critical impact on consumption rates of oxygen or ascorbic acid.

Only one type of pigment was identified in these experiments and further work is required to determine the identity of the other pigments formed and their mechanism of formation. The development of pigmentation under the conditions used here needs to be correlated with changes in the concentrations of the anti-oxidant components of the wine matrix, sulfur dioxide and ascorbic acid.

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